INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2007/09–5–726–730
http://www.fspublishers.org

# Physical, Chemical and Microbiological Changes during Storage of Orange Juices cv. Sai Nam Pung and cv. Khieo Waan in Northern Thailand

WISSANEE SUPRADITAREPORN[1] AND RENU PINTHONG
*Department of Food Science and Technology, Faculty of Agro-industry Chiang Mai University, Chiang Mai, Thailand*
[1]Corresponding author's e-mail: wissanee@hotmail.com

## ABSTRACT

The experiment was conducted to study the physical, chemical and microbiological properties of fresh orange juices cv. Sai Nam Pung and cv. Khieo Waan in Northern Thailand, immediately after harvest and different storage periods and storage temperatures. Some of the parameters could be used as indicator of quality loss of the juices such as colour L*, a* and b*, total soluble solid, titratable acidity, ascorbic acid and total plate counts varied with storage time and temperature. The shelf life of orange juices both cv. Sai Nam Pung and cv. Khieo Waan had only 1 day shelf life at 25°C, 6 days at 4°C and more than 21 days at -18°C. Sai Num Pung and Khaieo Waan at -18°C still have a good quality throughout the storage time; however, ascorbic acid contents were reduced.

**Key Words:** Orange juice; Sai nam pung; Khieo waan; Chemical change; Storage; Shelf life

## INTRODUCTION

Orange juice is one of the popular fruit juices in Thailand. Thai consumers like the juice, because of its high vitamin C content and the unique, delicate and desirable flavour. Orange juice is reported to be a source of carotenoids. Consumption of this vitamin has been correlated with a reduction in the incidence of certain cancers (Vinson *et al*., 2002). In Thailand, orange juice is usually purchased in glass jars, cans or flexible containers that are kept in refrigerators or in boxes containing ice cubes or at room temperature. On average, Thai people consume 3 L $head^{-1}$ $year^{-1}$ of all fruit juices. According to market research carried out in Thailand, use of fresh un-pasteurized orange juice has increased (Thailand Institute of Scientific & Technological Research, 2004). The recent trends show that the consumer preference towards un-pasteurized orange juice has increased due to its superior taste, aroma and nutritive values. However, the juice is less stable during storage and may become un-safe due to the growth of food-borne and spoilage bacteria. Microorganisms associated with the spoilage of orange juices have long been studied (Fellers, 1988; Parish, 1998; Farnworth *et al.*, 2001; Alwazeer *et al*., 2002; Johnston & Bowling, 2002).

Production of tangerines in Thailand can be done under a wide range of climatic conditions. The total production of citrus in Thailand was about 765,000 tones in 2000. Most of this production consumed by the domestic market, although a small quantity of pomelos was exported (Ketsa, 1990). A Khieo Waan and Sai Nam Pung (*Citrus reticulata* Blanco) variety of the tangerine occupies the largest growing area in Thailand and was produced as the highest yield citrus. The fruit has a short shelf life under the hot ambient temperature of tropical climates. Post harvest losses in citrus fruits have been observed too much due to which fruit loses its quality (Tariq *et al.*, 2001a). Orange juice quality depends on the raw material, processing conditions, storage conditions and packaging materials. The usual practice of washing of citrus fruits after harvest was an effective method for removing spores from the surface of fruits (Tariq *et al.*, 2001b).

Storage temperature was the prime limiting factor for shelf life of this product. Much work had been done on the development, pasteurization, characterization, and nutrition of orange juice but there is no report of the specific physical, chemical and microbiological properties of the fresh orange juices from cv. Sai Nam Pung and cv. Khieo Waan after production and during their storage periods at different temperatures.

## MATERIALS AND METHODS

Orange juice was prepared from fresh orange varieties Sai Nam Pung and Khieo waan. Samples were obtained from a private farm at Fang village, Chiang Mai province, Thailand during the 2005 - 06 harvest seasons. Random sample of a uniform size and colour were taken. Fresh oranges were hand-peeled and squeezed with stainless press for the juice. The orange juice was filtered to remove its pulp. Samples of the filtered juice were placed in a sterilized bottle and kept for all the experiments in this study.

Fresh orange juice was prepared at 3 different storage temperatures 4, -18°C and at room temperature (25°C). Subsequently, the orange juice was analyzed for chemical, microbiological and sensory properties. The juice was analyzed on 0, 3, 6, 9, 12, 15 days stored at 4°C and -18°C until 21 days and on the 0, 1, 2, 3, 4, 5, 6, 7 days for the first 7 days storage at room temperature (25 ± 2°C).

**Color determination.** The measurement of color was done with the CIE system L*, a*, b*. The orange juice color assessed by colourimeter (Minolta CR-300, Japan) for the values of L (lightness or darkness), a* (redness or greenness) and b* (yellowness or blueness).

**Chemical analysis.** Total soluble solids (TSS) of orange juices were measured by a hand refractometer (Atago, Japan) and corrected to the equivalent reading at 20°C (AOAC, 1995). The titratable acidity (TA) was measured by diluting 10 g of the orange juice with 250 mL distilled water and titrated against 0.1 N NaOH to a pH 8.1 as the end-point by using a pH meter (AOAC, 1995) and expressed in g acid per 100 mL based on citric acid. The β-carotene in orange juice was determined by HPLC (Method of food analysis, 2002) with an HPLC Series 1100 Agilent. Reducing sugars and ascorbic acid were determined by the methods of (AOAC, 2000). Measurements were done in triplicate.

Orange serum agar plate count was used for to total microbial population. This analysis was done by preparing the medium. One mL of an orange juice sample was added into a sterile petri dish. Twenty mL of the medium was added at 50°C to the plate. The sample and the medium were mixed properly. Afterward, the plate was incubated at 30°C and examined for 2 days and reported as a number of colony-forming units per mL of orange juices (Harrigan, 1998).

## RESULTS AND DISCUSSION

Physical and chemical properties revealed that fresh orange juice cv. Sai Nam Pung and Khieo Waan showed the values of L*: 27.45 and 37.18; a*: 1.20 and 2.58; b*: 13.88 and 23.80, respectively there were significant difference ($p < 0.05$). The Khieo Waan was brighter (L*) and darker yellow (b*) than the Sai Nam Pung (Table I). TSS, TA, reducing sugar and ascorbic acid of fresh orange juice cv. Sai Nam Pung and Khieo Waan showed non-significant difference ($p < 0.05$). They contained the values of reducing sugar (mg $100^{-1}$ g): 3.46 and 2.83; ascorbic acid (mg $100^{-1}$ g): 28.23 and 25.33, respectively. Sai Nam Pung was higher β-carotene (μg/100 g) than Khieo Waan significant difference ($p < 0.05$), They contained the values 42.73 and 19.85, respectively. In general the fresh orange juice cv. Sai Nam Pung showed the value of reducing sugar, ascorbic acid and β-carotene more than Khieo Waan. Similar results were observed for the data in the literature of Linchong (2003).

Monitoring the shelf life of the orange juices cv. Sai Nam Pung and cv. Khieo Waan during storage at room temperature (25°C), 4 and -18°C. Consider the limited for shelf life estimation of orange juice (Table II & III). The criteria of orange juices cv. Sai Nam Pung and Khieo Waan used for total plate count (TPC) was of 5 x $10^4$ CFU $mL^{-1}$ (Directive 92/46/EEC, 2006). Fresh orange juice had only one day shelf life. Furthermore, the orange juice qualities at 4, -18°C and room temperature were evaluated. It was observed that 4°C and -18°C, the orange juice still had a good quality throughout the storage time; however, ascorbic acid contents were reduced. So, it can be concluded from this trial that the end of storage time at each temperature condition should be in 1 day at room temperature, 6 days at 4°C and more than 21 days at -18°C. The number of microorganisms in orange fruits can affect the microbial quality of fresh orange juices. A specific research related to this issue was done to quantify the transfer rates of microorganisms during an extraction process. It was found that about 1.7 - 2.6% of total aerobic organisms and 2.3 - 2.6% of aciduric organisms from the washed oranges were introduced into the fresh juice during the extraction process. The quality of fresh juice is essentially depending on careful fruit handling and strict processing sanitation (Steven & Davis, 2001).

**Colour determination.** The L* (L* whiteness/darkness, ranged from 0 to 100) shown in Fig. 1, a* (a* redness for positive value and greenness for the negative one) shown in Fig. 2 and b* (b* yellowness for positive & blueness for negative value) shown in Fig. 3. The fresh Khieo Waan had a significant higher L*, a*, b* ($p < 0.05$) than the fresh Sai Nam Pung (Table I). Data showed that there was a steady trend in the L* values of the Khieo Waan storage at 4°C (K4) and Sai Nam Pung storage at 4°C (S4) for 6 days and the Khieo Waan storage at -18°C (K-18) for 21 days (Fig. 1 & 2), no variation in L* were seen (data not shown). During storage of orange juices cv. Sai Nam Pung and Khieo Waan at 4°C and -18°C, it was observed that b* parameters did not present significant variations until 15 days of storage (Fig. 3). After this time, b* slightly changed its color from initial yellow to red-dish tones. This change may be attributed to non-enzymatic browning (Linchong, 2003).

**Total soluble solid.** The results indicated that total soluble solid of fresh orange juice cv. Sai Nam Pung and Khieo Waan were not significant difference ($p < 0.05$) (Table I). During storage, orange juice both cv. Sai Nam Pung and Khieo Waan at 4°C had a same steady total soluble solid about 11.8% for 3 days and decrease until 6 days (Fig. 4). This decrease may be attributed to consumption of sugar as a result of the onset of fermentation. The K-18 and S-18 remained steady. It was found that total soluble solid were not significant difference ($p < 0.05$) (Data not shown). Both of them had similar TSS until storage at 21 days.

**Titratable acidity.** During storage of orange juice cv. Sai Nam Pung and Khieo Waan at -18°C, no variations in TA was evident. This indicated similar quality of two varieties in term of citric acid. However, at 4°C the acidity began to increase in 3 - 6 days (Fig. 5). The increase in TA indicated fermentation of orange juice. The low pH values of some orange juices greatly limit the number and types of bacteria that can survive or grow. Orange juices had pH between 3.4

**Table I. Physical and chemical properties of orange juices cv. Sai Nam Pung and cv. Khieo Waan**

| Assay | Sai Nam Pung | Khieo waan | Significant level |
|---|---|---|---|
| Colour L* | 27.45 ± 4.14 a | 37.18 ± 4.04 b | * |
| Colour a* | 1.20 ± 0.81 a | 2.58 ± 0.03 b | * |
| Colour b* | 13.88 ± 0.08 a | 23.80 ± 0.11 b | * |
| Total soluble solids (%) | 11.93 ± 0.06 | 11.46 ± 0.12 | NS |
| Total titratable acidity (%) | 0.65 ± 0.15 | 0.52 ± 0.17 | NS |
| Reducing sugar (mg $100^{-1}$ g) | 3.46 ± 0.17 | 2.83 ± 0.11 | NS |
| Ascorbic acid (mg $100^{-1}$ g) | 28.23 ± 0.25 | 25.33 ± 0.31 | NS |
| β-carotene (μg $100^{-1}$ g ) | 42.73± 0.15 b | 19.85± 0.09 a | * |

Values are means ± standard deviations.
Values followed by different letters are significantly different ($p < 0.05$).
NS = not significant

**Table II. Changes in Total Plate Count in orange juice during storage at room temperature (25°C), 4 and -18°C**

| | | Total Plate Count (CFU $mL^{-1}$) | |
|---|---|---|---|
| Temp. | Days | Sai Nam Pung | Khieo Waan |
| 25°C | 0 | 3.83x$10^2$ a | 4.10x$10^2$ a |
| | 1 | 4.91 x$10^3$ a | 4.37 x$10^3$ a |
| | 3 | 1.91 x$10^6$ a | 2.37 x$10^6$ b |
| 4°C | 0 | 3.86x$10^2$ a | 4.10x$10^2$ a |
| | 1 | 7.93 x$10^2$ a | 8.13 x$10^2$ a |
| | 3 | 1.06 x$10^3$ a | 1.09 x$10^3$ a |
| | 6 | 1.06 x$10^4$ a | 1.09 x$10^4$ a |
| | 9 | 1.77 x$10^6$ a | 1.94 x$10^6$ a |
| -18°C | 0 | 3.80x$10^2$ a | 4.16x$10^2$ a |
| | 1 | 5.53 x$10^2$ a | 5.50 x$10^2$ a |
| | 3 | 6.5x$10^2$ a | 6.4x$10^2$ a |
| | 6 | 7.1x$10^2$ a | 7.4x$10^2$ a |
| | 9 | 7.5x$10^2$ a | 7.8x$10^2$ a |
| | 12 | 8.1x$10^2$ a | 8.3x$10^2$ a |
| | 15 | 9.4x$10^2$ b | 8.3x$10^2$ a |
| | 18 | 9.8x$10^2$ b | 8.6x$10^2$ a |
| | 21 | 1.10 x$10^3$ b | 1.02 x$10^3$ a |

Values followed by different letters are significantly different ($p < 0.05$).

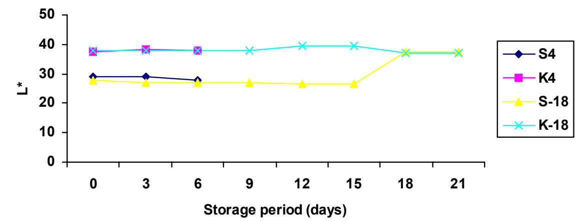
**Table III. Shelf life of orange juices cv. Sai Nam Pung and cv. Khieo Waan at room temperature (25°C), 4 and -18°C (based on microbiological)**

| Storage temperature (°C) | Shelf life(days) | |
|---|---|---|
| | Sai Nam Pung | Khieo Waan |
| 25 | 1 | 1 |
| 4 | 6 | 6 |
| -18 | > 21 | > 21 |

and 4.0 and in these conditions the lactic acid bacteria considered to be spoilage organisms causing the formation of slime, gas, off-flavor, turbidity and changes in acidity (Murdock & Hatcher, 1975).

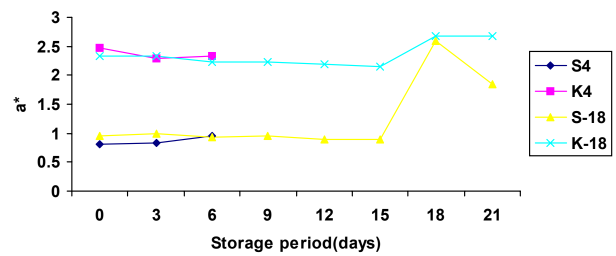
**Ascorbic acid.** Ascorbic acid of both varieties showed the decrease trend (Table IV), were significant ($p < 0.05$). The results from our experiment agree with previous reported Hoare *et al*. (1993). In orange juices, the ascorbic acid levels significantly decreased during a storage period even in un-opened containers. Therefore, the presence of dehydroascorbic acid needed to be measured besides the ascorbic acid to get the overall activity of the vitamin C in orange juices. The content of vitamin C in orange juices was in a range of 26 to 84 mg $100^{-1}$ mL (Ashurst *et al*., 1999). Unfortunately, the concentration of this vitamin was reduced during storage of the juices (Li *et al*., 1989; Lee & Coates, 1999; Johnston & Bowling, 2002). For example, vitamin C in fresh squeezed un-pasteurized orange juices was declined during a frozen storage in a polyethylene bottle at -23°C (Lee & Coates, 1999). Li *et al*. (1989) also found the concentration of vitamin C in Valencia orange

**Fig. 1. Changes in colour L* in orange juice cv. Sai Nam Pung and cv. Khieo Waan during storage at 4°C and -18°C**



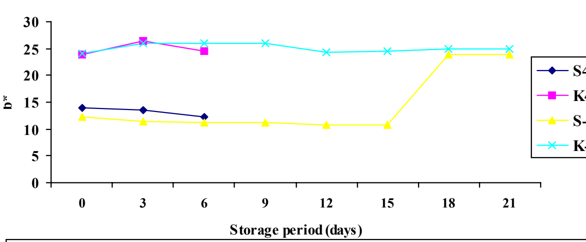

Sai Nam Pung storage at 4°C (S4), Sai Nam Pung storage at -18°C (S-18)
Khieo Waan storage at 4°C (K4), Khieo Waan storage at -18 °C (K-18)

**Fig. 2. Changes in colour a* in orange juice cv. Sai Nam Pung and cv. Khieo Waan during storage at 4°C and -18°C**




Sai Nam Pung storage at 4°C (S4), Sai Nam Pung storage at -18°C (S-18)
Khieo Waan storage at 4°C (K4), Khieo Waan storage at -18 °C (K-18)

**Fig. 3. Changes in colour b* in orange juice cv. Sai Nam Pung and cv. Khieo Waan during storage at 4°C and -18°C**



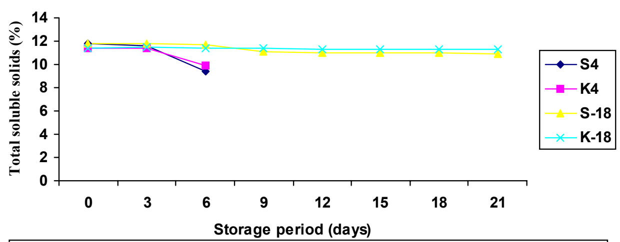

Sai Nam Pung storage at 4°C (S4), Sai Nam Pung storage at -18°C (S-18)
Khieo Waan storage at 4°C (K4), Khieo Waan storage at -18 °C (K-18)

**Table IV. Changes in ascorbic acid in orange juice cv. Sai Nam Pung and cv. Khieo Waan during storage at room temperature (25$^{o}$C), 4 and -18$^{o}$C**

| Methods | Ascorbic acid (mg $100^{-1}$ g) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0 days | 1 days | 3 days | 6 days | 9 days | 12 days | 15 days | 18 days | 21 days |
| $S_{rt}$ | 28.23$^{b}$±0.25 | 18.43$^{a}$ ± 0.58 | ND | ND | ND | ND | ND | ND | ND |
| $K_{rt}$ | 25.33$^{a}$±0.31 | 14.60$^{a}$ ± 0.60 | ND | ND | ND | ND | ND | ND | ND |
| S4 | 28.23$^{b}$±0.25 | 25.83$^{c}$ ±0.12 | 25.63$^{b}$±0.30 | 9.93$^{b}$±0.40 | ND | ND | ND | ND | ND |
| K4 | 25.33$^{a}$±0.30 | 19.67$^{a}$ ±0.18 | 18.93$^{a}$±0.80 | 8.40$^{a}$±0.98 | ND | ND | ND | ND | ND |
| S-18 | 28.23$^{b}$±0.25 | 25.63$^{c}$ ±0.55 | 25.33$^{b}$±1.42 | 20.43$^{c}$±0.15 | 20.36 ±5.77 | 19.06 ± 0.60 | 16.73 ±2.88 | 14.23 ±0.12 | 11.66 ± 0.58 |
| K-18 | 25.33$^{a}$±.30 | 21.33$^{b}$ ±5.77 | 21.06$^{a}$±1.90 | 10.26$^{b}$±0.65 | 10.23 ± 5.77 | 9.53 ±0.31 | 8.00 ± 4.46 | 7.33 ±5.77 | 6.26 ±0.21 |

ND = not detectable (spoilage)
Values are means ± standard deviations for triplicate measurements
Values followed by different letters are significantly different ($p < 0.05$).
Sai Nam Pung storage at room temperature 25°C (Srt)
Khieo Waan storage at room temperature 25°C (Krt)
Sai Nam Pung storage at 4°C (S4), Sai Nam Pung storage at -18°C (S-18)
Khieo Waan storage at 4°C (K4), Khieo Waan storage at -18 °C (K-18)

**Fig. 4. Changes in total soluble solids (%) in orange juice cv. Sai Nam Pung and cv. Khieo Waan during storage at 4$^{o}$C and -18$^{o}$C**




Sai Nam Pung storage at 4°C (S4), Sai Nam Pung storage at -18°C (S-18)
Khieo Waan storage at 4°C (K4), Khieo Waan storage at -18 °C (K-18)

juices was decreased during storage at 5 and 25$^{o}$C. Beside these researches, Kabasakalis *et al.* (2000) found that if the juices stored in open containers in a refrigerator for 31 days, the ascorbic acid loss was around 60 to 67%.

## CONCLUSION

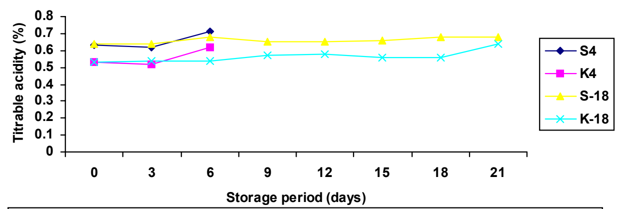
Storage temperature was the prime limiting factor for shelf life of orange juice. However, various effects of low temperatures on the keeping quality of the fresh orange juices prepared from oranges cv. Sai Num Pung and Khieo Waan can make freshly orange juice economically feasible to market. The shelf life of the natural fresh orange juices stored at room temperature (25$^{o}$C), 4$^{o}$C and -18$^{o}$C could be extended only 1, 6 and more than 21 days, respectively because beyond these periods, both their total colony counts were higher than the above standard limit and their odor and chemical changes were unacceptable.

**Acknowledgement.** I thank Graduated School, Department of Food Science and Technology, Faculty of Agro-industry Chiang Mai University for providing laboratory facilities.

## REFERENCES

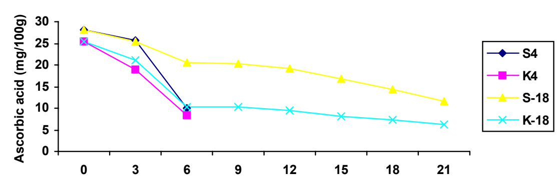
Alwazeer, D., R. Cachon and C. Divies, 2002. Behavior of Lactobacillus plantarum and Saccharomyces cerevisiae in fresh and thermally processed orange juice. *J. Food Protect.*, 65: 1586–9

**Fig. 5. Changes in titratable acidity (%) in orange juice cv. Sai Nam Pung and cv. Khieo Waan during storage at 4$^{o}$C and -18$^{o}$C**




Sai Nam Pung storage at 4°C (S4), Sai Nam Pung storage at -18°C (S-18)
Khieo Waan storage at 4°C (K4), Khieo Waan storage at -18 °C (K-18)

**Fig. 6. Changes in ascorbic acid (mg/100g) in orange juice cv. Sai Nam Pung and cv. Khieo Waan during storage at 4$^{o}$C and -18$^{o}$C**




Sai Nam Pung storage at 4°C (S4), Sai Nam Pung storage at -18°C (S-18)
Khieo Waan storage at 4°C (K4), Khieo Waan storage at -18 °C (K-18)

AOAC, 1995. *Official Method of Analysis of AOAC International*, 16$^{th}$ edition. The United States of America
AOAC, 2000. *Official Method of Analysis of AOAC International*, 17$^{th}$ edition. The United States of America
Ashurst, P.R., 1999. *Production and Packaging of Non-carbonated Fruit Juices and Fruit Beverages,* 24$^{th}$ edition. Aspen Publish, Gaithersburg Maryland, USA
Farnworth, E.R., M. Lagacea, R. Coutureb, V. Yaylayan and B. Stewarta, 2001. Thermal processing, storage conditions and the composition and physical properties of orange juice. *Food Res. Int.*, 34: 25–30
Fellers, P.J., 1988. Shelf life and quality of freshly squeezed un-pasteurised polyethylene-bottled citrus juice. *J. Food Sci.*, 53: 1699–702
Harrigan, W.F., 1998. *Laboratory Methods in Food Microbiology,* 3$^{rd}$edition. Academic Press Limited, London
Hoare, M., S. Jones and J. Lindsay, 1993. Total vitamin C analysis of orange juice. *Food Australia*, 45: 341–5

Johnston, C.S. and D.L. Bowling, 2002. Stability of ascorbic acid in commercially available orange juices. *J. American Dietetic Assoc.,* 102: 525–9

Ketsa, S., 1990. Weight loss of tangerines as affected by peel thickness. *Tropical Sci.*, 30: 73–6

Kabasakalis, V., D. Siopidou and E. Moshatou, 2000. Ascorbic acid content of commercial fruit juices and its rate of loss upon storage. *Food Chem.,* 70: 325–8

Lee, H.S. and G.A. Coates, 1999. Vitamin C in frozen fresh squeezed unpasteurised polyethylene bottle orange juice: a storage study. *Food Chem.*, 65: 165–8

Li, Z., I. Alli and S. Kermasha, 1989. Use of acidification, low temperature and sorbates for storage of orange juice. *J. Food Sci.,* 54: 674–8

Linchong, S., 2003. Preservation of orange juice by combined application of mild heat and ginger extract. *Food,* 33: 211–20

Murdock, D. and W. Hatcher, 1975. Growth of microorganisms in chilled orange juice. *J. Food Technol.*, 38: 393–6

Parish, M., 1998. Orange juice quality after treatment by thermal pasteurisation or isostatic high pressure. *Lebensmittel-Wissen. Technol.,* 31: 439–42

Steven, P. and C.L. Davis, 2001. Transfer of natural and artificially inoculated microorganisms from orange fruit to fresh juice during extraction. *Lebensmittel-Wissenschaft and Technologie*, 34: 113–7

Tariq, M.A., F.M. Tahir, A.A. Asi and M.A. Pervez, 2001a. Effect of controlled atmosphere storage on damaged citrus fruit quality. *Int. J. Agric. Biol.*, 3: 9–12

Tariq, M.A., F.M. Tahir, A.A. Asi and J. Iqbal, 2001b. Effect of washing and seal packaging on scuffing damaged citrus fruit quality. *Int. J. Agric. Biol.*, 3: 461–3

Thailand Institute of Scientific and Technological Research, 2004. *Fruit juices*. Available from: http://www.tistr-foodprocess.com/ fruit.htm. Accessed on April 28, 2004

Vinson, J.A., X. Liang, J. Proch, B.A. Hontz, J. Dancel and N. Sandone, 2002. Polyphenol antioxidants in citrus juices *in vitro* and *in vivo* studies relevant to heart disease. *Adv. Experim. Medi. Biol.*, 505: 113–22

**(Received 09 January 2007; Accepted 12 April 2007)**